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Microbial nucleic acids employed in diagnostics, sequencing and phylogenetics are subject to detrimental inhibitors and mutagens

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Abstract

Sequencing the genomes of microbial species has increased tremendously. Nucleic acids (NA) are used also for diagnostic and phylogenetic analyses of microbes. It is essential that protocols ensure representative NA. The effect of the "spent" growth medium on NA has not been considered. Surprisingly, fungi are grown on media that support inhibitors and mutagens when producing NA for these purposes[1,2,3]. This situation is illogical as these secondary metabolites may affect the structure of NA[2] and/or inhibit PCR polymerases used in PCR[3].

The objective of the work was to highlight how NA analyses could be affected by self produced mutagens and inhibitors. Hence, (a) PCR of the *idh* gene of patulin production in fungi (e.g. *Penicillium expansum*) and (b) interpretation of the scientific literature were employed to determine the seriousness of the situation. Analysis of *idh* was successful for culture dependant PCR (CDP) and culture independent PCR (CIP). A reversible inhibition was observed in CDP presumably from inhibitors in cultures. Inhibition was observed in CIP. In some cases, taxa which were predicted to be positive for *idh* were not, and *vice versa*. A logical interpretation of this was that the gene was mutated by cultural components. In addition, the PCR reaction may have been inhibited and internal amplification controls (IAC) are required.

The conclusions were that it is illogical to grow microbes for NA analysis in a milieu of mutagens and inhibitors. Reports on diagnostic methods and phylogenetic schemes are undermined consequently. Work on *Aspergillus flavus* ismost vulnerable to this criticism, as they produce aflatoxins which are the most carcinogenic natural compounds. Numerous fungi produce inhibitors and mutagens and so the problem is widespread. There may be an equivalent situation for bacteria[2,3]. It is essential to grow microbes in a manner to avoid mutagens and inhibitors. Some recommended procedures would be to grow the cultures for a shorter period, although the ratio of mutagen to NA is important. Analysing cultures immediately upon isolation is preferred. Continuous culture could be used to avoid secondary metabolism. Finally, IAC are required for PCR in general.

1. Paterson, RRM. (2004) Mycol Res, 108, 1431-1437.
2. Paterson, RRM, Lima, N. (2009) J Appl Microbiol, 106, 1070–1080.
3. Paterson RRM. (2007) J Appl Microbiol, 102, 1-10.